Original Research Article

Low Notch-1 and high Jagged-1 expressions are associated with better treatment response and survival in Adult Egyptian Patients with Acute Myeloid Leukemia

Abstract

Background: Acute myeloid leukemias (AMLs) are neoplastic proliferations arising in hematopoietic precursor cells in the bone marrow resulting in overgrowth of myeloblasts and other cells of myeloid lineage. Notch-1 receptor is a transmembrane protein of type I. Interactions between Notch-1 and its ligands Jagged-1 and Dll-1 result in proteolytic cleavages inside the receptor, followed by the release and nuclear translocation of the intracellular domain (Notch-1-IC). Notch-1 expression was also found in CD34+ bone marrow progenitors and other cells in peripheral blood and bone marrow. Jagged-1 and Delta-like1 (Dll-1) seem to be functionally opposing members of the Notch-1 ligand family; however, their precise methods of action in AML are unknown.

Methods: Using flow cytometry, the expression of the Notch-1 intracellular domain and the surface expression of Jagged-1 and Dll-1 ligands on leukemic blasts from newly diagnosed AML patients was evaluated. In addition, protein expression was associated with clinical data, laboratory data, responsiveness to therapy, disease-free (DFS) and overall survival (OS).

Results: Notch-1 was positively expressed in 20% of studied patients. Positive Notch-1 expression was associated with shorter OS (6.1 months) and DFS (3.1 months). Higher Notch-1 expression levels were significantly associated with lower remission and higher relapse rates. In contrary, Jagged-1 protein marker weas positively expressed in 56% studied patients, those patients showed shorter OS (6.6 months) and longer DFS (3.6 months) compared to negatively expressed ones. Higher Jagged-1 expression levels were significantly associated with higher remission and lower relapse rates. Positive Dll-1

expression was recorded in 30 % patients, yet with no significant relationship with OS and DFS rates as well as clinical outcome after therapy.

Conclusion: Both Notch-1 receptor and its ligands, Jagged-1 and Dll-1, seem to be implicated in AML pathogenesis, however Jagged-1 has a greater impact on clinical findings than Dll-1. A better prognosis is related with high Jagged-1 surface expression in individuals with AML. Hence, further research is required to acquire a greater knowledge of the modes of action of Notch-1 and its ligands in AML.

Keywords

Leukemia; AML; Notch; Flow cytometry.

Introduction

"Acute myeloid leukemia (AML) is the most prevalent adult malignant myeloid disease, defined by the inhibition of myeloid development and the buildup of blast cells in the bone marrow" (Kumar, 2011).

"Excluding promyelocytic acute leukemia, one of the greatest obstacles in AML is the high recurrence rate following treatment due to the presence of remaining blast cells in the bone marrow" (Zhang et al., 2013) (Ito et al., 2013).

"Notch is a physiological and adaptive mechanism that regulates normal and cancer cellular growth, self-renewal, distinctions, and survivability. Notch signalling has four receptors, Notch1 through Notch4, and five ligands, namely Jagged1, Jagged2, Dll1, Dll3, and Dll4" (South et al., 2012).

"Some studies have documented Notch expression and stimulation in AML specimens and AML cell lines; however, the mechanism was only weakly active, as shown by the low expression level of Notch target genes" [11-13]. "Similarly, they observed that reactivating Notch signalling caused blast cells to undergo apoptosis and differentiate into mature cells" (Lobry et al., 2013) (Kannan et al., 2013) (Tohda et al.,2005)

"In contrary, Notch activation mechanism in AML has been linked with a poor prognosis" (Xu et al, 2011). "Furthermore, individuals with Notch1 hyperexpression had

a worse mortality risk" (Sliwa et al, 2014). "Recent research by Grieselhuber and colleagues identifies Notch expression and activity in acute promyelocytic leukemia and demonstrates that suppression of Notch signaling genetically and pharmacologically suppresses the increased self-renewal of blast cells" (Grieselhuber et al, 2013). Conversely, the influence of exogenous microenvironmental Notch signaling on the AML cells survival and their sensitivity to treatment has not yet been determined.

"Jagged-1 and Delta-like 1 (Dll-1) appear to be physiologically opposing siblings of the Notch-1 ligand family, although their precise roles in AML remain uncertain" (Chiaramonte et al, 2005) (Tohda et al, 2003).

So, this research was performed to help understanding the role of Notch-1, Jagged-1 and Dell-1 in treatment response and prognosis of AML.

Patients and methods

The present research involved 50 subjects with newly diagnosed AML. Cases presented to the Hematology/Oncology unit, Internal Medicine department, Tanta University Hospital and Tanta Oncology Institute. The studied subjects were 30 males and 20 females with male to female ratio 1.5:1 and their ages ranged from 48 to 61 years.

Inclusion criteria: Subjects older than 18 years newly diagnosed with AML.

Exclusion criteria: Subjects previously diagnosed with AML and receiving treatment, or patients with any malignant disease other than AML were excluded from the study.

All subjects underwent full history taking and complete clinical investigation, routine laboratory investigations: CBC including differential count (by ERMA PCE-210N cell counter with examination of peripheral blood films stained with Giemsa stain), LDH, liver enzymes tests**,** bone marrow aspirates (BMA) were taken and examined for morphology, cytochemical stain and immunophenotyping. Specific laboratory tests include: Flow cytometric analysis of the bone marrow aspirate samples to detect Notch-1, Jagged-1 and Dll-1 expression on leukemic blast cells.

After a thorough evaluation, conventional induction chemotherapy with daunorubicin and cytarabine was administered to all (80%) subjects. Unfit elderly individuals were treated

with repeated sessions of decitabine or low dose cytarabine (20%). All trans retinoic acid (ATRA) was added to patients with promyelocytic leukemia (M3).

Follow up of the patients

The patients were observed for 18 months to determine the overall survival (OS) and disease-free survival (DFS).

OS is calculated from the date of diagnosis to the date of death from any cause; patients not known to have died at the time of the most recent follow-up are censored on the day they were last known to be alive.

DFS is calculated from the date of the end of induction to the date of relapse or death from any cause; patients not known to have relapsed or died at the time of the last examination are censored on the date of the last examination.

The differences in survival were analyzed utilizing the Kaplan-Meier method

Sampling

Two ml of peripheral venous blood were collected into an EDTA vacutainer tube for complete blood count and Giemsa-stained smears and were labelled. One ml of peripheral blood was delivered into EDTA vacutainer tube and used for immunophenotypic determination. Two ml blood were collected into an empty tube and serum was separated for measurement of serum LDH and liver and renal tests.

Flow cytometry of targeted proteins

1. Procedure for intracellular marker (NOTCH-1):

For each sample, two tubes were labeled, one for negative isotopic control and the other for intracellular marker NOTCH-1. The cell count was adjusted to10^6. The next steps were done to each sample. 100 µl pf peripheral blood were put in the staining tube. Adding of 2 ml of FACS lysing solution. The tubes were vortexed and incubated in the dark at room temperature for 15 minutes. Spinning down the samples at 1400 rpm for 5 minutes. Discard the supernatant and save the pellets. Adding of 2 ml of phosphate buffered saline (PBS). Spinning down the samples at 1300 rpm for 5 minutes. Discard the supernatant and save the pellets. Adding of 400 µl of BD permilization solution (BD Cytofix/CytopermTM) for each sample. Incubation in the dark at room temperature for 15 minutes. Adding of 2 ml of washing buffer (PBS). Spinning down the samples at 1300 rpm for 5 minutes. Discard the supernatant and save the pellets. Resuspension of the pellets in 100 µl of PBS. Adding of the aliquots of the antibody, Anti-NOTCH-1 monoclonal antibody PE labeled (BD biosciences), Catalogue number (CN) 560972, clone 9F10, (5 µl to the sample tube) and mix well. Incubation in the dark at room temperature for 30 minutes. Adding of 2 ml of PBS. Spinning down the samples at 1300 rpm for 5 minutes. Discard the supernatant and save the pellets. Resuspension of the pellets in 500 µl of PBS. Run the samples in the cytometer (Acquisition).

2. Procedure for cytoplasmic markers (Jagged-1 and Dll-1):

For each sample, two tubes were labeled, one for negative isotopic control and the other for the monoclonal antibodies. The cell count was adjusted to 10^6. The next steps were done to each sample. 100 µl pf peripheral blood were put in the

staining tube. Adding the aliquots of the antibodies Anti-JAGGED-1 monoclonal antibody PE labeled (Abcam), Catalogue number (CN) 139943, clone T22-A, anti DLL-1 monoclonal antibody PE labeled (BD biosciences), Catalogue number (CN) 340576, clone 100, and PE labelled mouse isotopic negative control to prevent the nonspecific binding of monoclonal antibodies (background fluorescence intensity). (5 µl of each antibody) to the blood and mix well. Incubation in the dark at room temperature for 30 minutes. Adding of 2 ml of FACS lysing solution. Incubation in the dark at room temperature for 15 minutes. Spinning down the samples at 1400 rpm for 5 minutes. Discard the supernatant and save the pellets. Adding of 2 ml of PBS. Spinning down the samples at 1300 rpm for 5 minutes. Discard the supernatant and save the pellets. Resuspension of the pellets in 500 µl of PBS. Run the samples in the cytometer (Acquisition).

Flow cytometric analysis

Becton Dickinson's FACS flow cytometry was used for analysis. Cell search software was utilised for automated data collection and analysis. The equipment was calibrated using manufacturer-supplied calibrated beads. Using isotopic quality control, nonspecific binding and autofluorescence were ruled out. 10.000 events (cells) at least were passed in front of the laser for each case from which the blast cells were selectively gated (surrounded by a line to separate them from other cells in the basic histogram) for immunophenotyping analysis. Forward light scatter vs log side scatter histogram was utilised to identify cell populations of interest (Myeloblasts) using bitmap sketching (gating). In order to define 98 percent of positive cells, the cursor position from the dot plot for isotopic controls is used to assess the gated fluorescence dot plot for positive cells.

Interpretation of the results

After 10000 events were counted, the numbers of blast cells expressing the markers emitting fluorescence signals were summated and multiplied in the PMT2 and the computer analyzed the data as a single-colored frequency histogram.

A case was defined as Notch-1, Jagged-1 or Dll-1 positive if more than or equal to 30% of the gated cells expressed the marker.

Statistical analysis

Statistical presentation and analysis of the study were conducted using the IBM SPSS Statistics Version 25 (SPSS Inc., Chicago, Ill., USA).

Numeric data was presented as mean and standard deviation, while categorical data as number and percentage. The distributions of numeric variables were tested for normality also histogram and QQ plot were used for vision test.

For normally distributed numeric data, Student *t* test was used to compare means of two independent variables. However, Mann Whitney U test was used for non-normally distributed variables. One-way ANOVA test was applied for mean comparison between more than two independent groups of parametric data. However, Kruskal-Wallis test was the alternative for non-parametric data. For categorical data, and chi-square or Fisher's exact tests were used to compare their frequencies.

Correlations between two quantitative variables were assessed using *Pearson* coefficient (*r*). Binary logistic regression, a predictive analysis to examine relationship between numeric independent variables and dichotomous (binary) dependent variable.

Survival analysis

Equality of survival between studied groups was tested by using log rank test, after plotting of data on Kaplan-Meier curve.

Significance test results were quoted as two-tailed probabilities. *P*-value $\leq .05$ was considered significant.

Results

Clinically, in spite of higher percentage of fever, infection, hepato-splenomegaly, and lymphadenopathy in Notch-1 +ve patients, the difference was not statistically significant. Only TLC was significantly higher in Notch-1 +ve patients when compared to Notch –ve patients. On the other hand, bleeding was significantly more common in Jagged-1 positive patients as shown in (Table 1).

When we studied the different FAB classes in relation to the studied parameters, we could not find a significant association (Table 2).

Interestingly, there was a better treatment response in patients with lower expression of Notch-1, while that was the case with higher expression of Jagged-1. (Table 3).

Binary logistic regression models were performed to predict the potential effect of biomarkers expression on clinical outcome and establish if there's -statistically significant- relationship between these variables. It was noticed that increased notch-1 expression was associated with worse clinical outcome; lower odds of CR (OR = 0.92) and marginally higher odds of relapse (OR = 1.04). However, Jagged-1 was associated with better outcome; higher odds of CR (OR = 1.06) and decreased odds of relapse (OR = 0.96). Nevertheless, Dll-1 expression level proved to non-significant predictor for neither complete remission nor relapse. (Table 4)

On studying OS and DFS, we found that both rates revealed non-significant difference between Notch-1, Jagged-1 and Dell-1 in positive and negative expression groups (p-value= 0.69, 0.54, 0.77 for OS and 0.20, 0.08, 0.64 for DFS) respectively. (**Error! Reference source not found.**1).

Table 1: Clinical and laboratory data of AML patients in relation to Notch-1, Jagged-1 and Dll-1 expression

Parameter	Notch-1			Jagged-1			Dll-1		
	+ve Notch-1 (n=10)	-ve Notch-1 (n=40)	p	+ve Jagged-1 (n=28)	-ve Jagged-1 (n=22)	p	+ve Dll-1 (n=15)	-ve Dll-1 (n=35)	p
Fever / Infection	9 (90%)	29 (72.5%)	0.27	18 (64.3%)	20 (90.9%)	0.2	8 (53.3%)	30 (85.7%)	0.11
Bleeding	8 (80%)	36 (90%)	0.60	27 (96.4%)	17 (77.2%)	0.02*	13 (86.7%)	31 (88.6%)	0.98
HSM	5 (50%)	22 (55%)	0.38	15 (53.5%)	12 (54.5%)	0.39	7 (46.7%)	20 (57.1%)	0.71
LNs	6 (60%)	16 (40%)	0.44	13 (46.5%)	9 (40.9%)	0.3	8 (53.3%)	14 (40%)	0.3
TLC ($x10^9$/L)	60.1 ± 24.8	39.5 ± 30.4	0.05*	45.79 ± 33.53	40.9 ± 26.1	0.57	44.1 ± 38.5	43.4 ± 26.7	0.94
Hb (gm/dL)	8.8 ± 1.9	9.6 ± 1.8	0.18	9.19 ± 1.89	9.8 ± 1.7	0.26	9.4 ± 2.0	9.5 ± 1.7	0.88
Platelet count ($x10^9$/L)	122.8 ± 71.6	123.8 ± 60.2	0.96	121.89 ± 48.21	125.8 ± 77.0	0.84	125.7 ± 49.8	122.7 ± 67.0	0.88
Peripheral Blasts (%)	27.7 ± 15.6	25.2 ± 23.2	0.75	28.68 ± 21.99	21.9 ± 21.4	0.28	31.9 ± 24.9	23.0 ± 20.1	0.19
BM Blasts (%)	57.3 ± 19.3	51.6 ± 23.9	0.49	57.32 ± 21.92	46.9 ± 23.5	0.11	55.5 ± 25.8	51.5 ± 21.9	0.58
ALT (IU/L)	46.9 ± 36.3	45.1 ± 30.7	0.87	47.32 ± 34.48	43.0 ± 27.9	0.64	43.9 ± 30.5	46.1 ± 32.3	0.82
AST (IU/L)	89.6 ± 67.2	76.3 ± 40.5	0.42	82.39 ± 53.31	74.6 ± 36.9	0.56	78.2 ± 44.4	79.3 ± 48.0	0.94
LDH (U/L)	817.9 ± 432.7	847.8 ± 493.9	0.86	851.28 ± 518.98	829.8 ± 432.5	0.88	992.7 ± 525.8	777.1 ± 448.8	0.17
Uric Acid (mg/dL)	7.3 ± 1.3	7.5 ± 1.6	0.76	7.40 ± 1.61	7.5 ± 1.4	0.92	7.6 ± 1.5	7.4 ± 1.5	0.64

AST: Alanine aminotransferase. **AST**: Aspartate transaminase. **BM**: bone marrow. **Hb**: hemoglobin. **HSM**: hepato-splenomegaly. **LDH**: lactic dehydrogenase. **LN**: lymphadenopathy. **TLC**: total leucocytic count.

Table 2: FAB classification in relation to Notch-1, Jagged-1 and Dll-1 expression

M stage	Notch-1			Jagged-1			Dll-1		
	+ve / -ve	Expression (Mean ± SD)	p	+ve / -ve	Expression (Mean ± SD)	p	+ve / -ve	Expression (Mean ± SD)	p
M0 (n=1)	0 /1	21.00	0.99	1 /0	35.00	0.29	0 /1	8.00	0.65
M1 (n= 2)	0 /2	21.50 ± 7.78		2 /0	44.50 ± 4.95		1 /1	33.00 ± 15.56	
M2 (n=13)	3 /10	28.46 ± 23.47		4 /9	25.08 ± 22.59		5 /8	26.77 ± 18.48	
M3 (n= 5)	1 /4	20.80 ± 5.63		4 /1	56.80 ± 24.71		3 /2	33.40 ± 21.82	
M4 (n= 8)	1 /7	26.25 ± 23.38		6 /2	42.75 ± 20.82		1 /7	21.75 ± 14.76	
M5 (n=16)	4 /12	27.13 ± 25.49		9 /7	41.25 ± 27.28		3 /13	21.00 ± 12.55	
M6 (n=5)	1 /4	24.20 ± 26.40		2 /3	30.60 ± 28.80		2 /3	27.00 ± 21.00	

Table 3: AML outcome in relation to Notch-1, Jagged-1 and Dll-1 expression

Treatment Response		Notch-1				Jagged-1				Dll-1			
		+ve	-ve	Expression	p	+ve	-ve	Expression	p	+ve	-ve	Expression	p
Complete Remission	**Yes (n=21)**	2 (9.5%)	19 (90.5%)	15.3 ± 8.0	0.001*	13 (61.9%)	8 (38.1%)	53.4 ± 25.5	0.001*	8 (38.1%)	13 (61.9%)	26.8 ± 16.6	0.44
	No (n= 29)	8 (27.6%)	21 (72.4%)	33.8 ± 25.4		15 (51.7%)	14 (48.3%)	26.5 ± 17.8		7 (24.1%)	22 (75.9%)	23.1 ± 16.3	
Relapse	**Yes (n=15)**	5 (3.3%)	10 (66.7%)	38.3 ± 27.8	0.04*	6 (40%)	9 (60%)	24.5 ± 17.5	0.04*	3 (20%)	12 (80%)	22.3 ± 17.0	0.51
	No (n=35)	5 (14.3%)	30 (85.7%)	20.8 ± 16.7		22 (62.9%)	13 (37.1%)	43.5 ± 25.9		12 (34.3%)	23 (65.7%)	25.7 ± 16.1	
Death	**Yes (n=14)**	3 (21.4%)	11 (78.6%)	29.1 ± 22.5	0.55	9 (64.3%)	5 (35.7%)	28.5 ± 18.5	0.06	4 (28.6%)	10 (71.4%)	24.0 ± 16.1	0.86
	No (n=36)	7 (19.4%)	29 (80.5%)	24.9 ± 21.9		19 (52.8%)	17 (47.2%)	41.4 ± 26.5		11 (30.6%)	25 (69.4%)	24.9 ± 16.6	

Table 4: Logistic regression of the studied biomarkers expression level in complete remission and relapse:

Response	Biomarkers expression level %	Odds Ratio	95% CI	P-value
CR	Notch-1	0.92	0.86 - 0.99	0.03*
	Jagged-1	1.06	1.02 - 1.09	0.001*
	Dll-1	1.01	0.98 - 1.05	0.43
Relapse	Notch-1	1.04	1.01 - 1.07	0.02*
	Jagged-1	0.96	0.94 - 0.99	0.02*
	Dll-1	0.99	0.95 - 1.03	0.50

CI: confidence interval. CR: complete remission. P: p value.

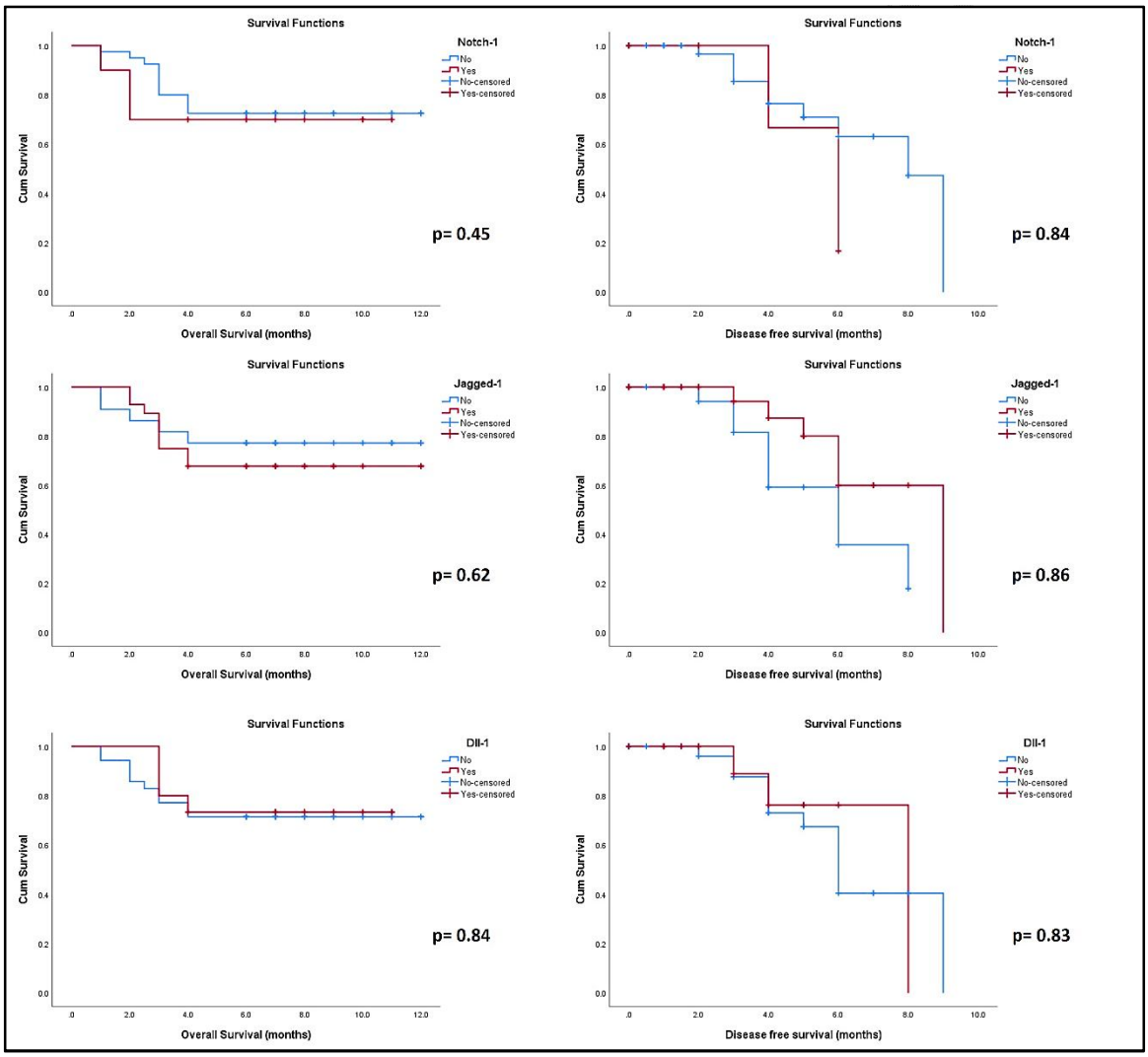

Figure 1: Kaplan-Meier curves for overall survival and disease-free survival in relation to Notch-1, Jagged-1 and Dll-1 expression

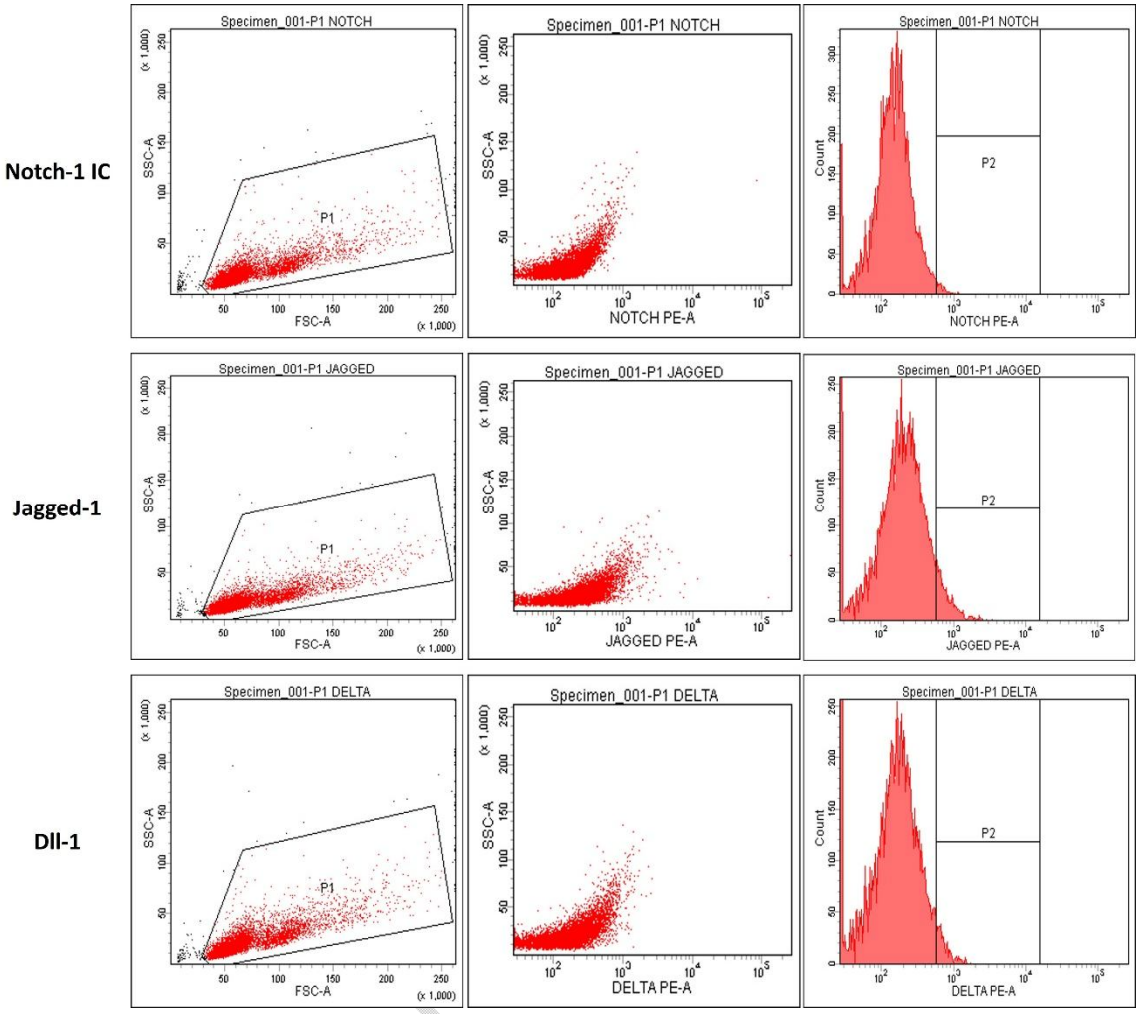

Figure 2: Expression patterns of Notch-1-IC, Jagged-1 and Dll-1 in AML blasts

Discussion

"AML is a significant obstacle for individualized medication. Due to the combination of modern diagnostic methods, such as genome-wide molecular profiling, immunophenotyping, cytogenetic, and clinical characteristics, several biomarkers guiding therapy approach have been identified" **(**Prada-Arismendy et al, 2017)

"Notch receptors are single-pass transmembrane proteins that play a crucial role in determining the destiny of cells and have been implicated in the control of several developmental events. Notch receptors transmit signals across short distances by

engaging with transmembrane Delta-like and Jagged ligands on adjacent cells". (Gragnani et al, 2021)

"In addition, the significance of aberrant Notch signaling in hematological malignancies has emerged as an intriguing area of cancer research. Over 50% of T-cell acute lymphoblastic leukemias have oncogenic Notch1 receptor mutations (T-ALL)" (Weng et al, 2004). Intriguingly, in the case of AML, the involvement of the Notch pathway is poorly known, with contradictory outcomes described in various papers.

In AML the role of Notch remains controversial (Takam et al, 2021). Kannan et al. identified a modest activation of the Notch system, as shown by the low level of Notch target gene expression. (Kannan et al, 2013) Similarly, Lobry et al. (2013) identified "epigenetic suppression of Notch target genes in AML, demonstrating that restoration of Notch signaling caused death and differentiation of leukemia blast cells into mature cells. These findings support the anti-leukemic function of demethylating/hypomethylating drugs azacytidine or decitabine in AML" (DiNardo et al., 2018; Leung et al., 2019). "However, other researchers discovered that Notch activation among AML samples and cell lines is not uniform" (Sliwa et al., 2014; Czemerska et al., 2014).

Hereby, in this study we aimed to evaluate the clinical role of Notch-1, Jagged-1 and Dll-1 in 50 adult patients newly diagnosed as AML. We have searched for the potential relationship between expression of these biomarkers in one hand and clinical presentation, certain laboratory findings, and clinical outcomes as well as survival rates after therapy. The expression pattern of Notch-1-IC, Jagged-1 and Dll-1 was represented by the multicolored fluid cytometry in AML cases.

Notch-1 was positively expressed in only 20% of the cases at mean expression level of 64.10 ± 19.65 % and negatively expressed in the remaining 80% with mean expression level of 16.55 ± 7.01 %. While Jagged-1 and Dll-1 markers were positively expressed in 56% and 30% and negatively expressed in 44% and 70% of cases, respectively. Their mean positive expression levels were 56.14 ± 17.07 % and 47.13 ± 7.03 %, respectively.

"Different expression pattern was recorded by Czemerska et al., who analyzed the expression examined proteins (Notch-1, Jagged-1, Dll-1) in leukemic cells and peripheral

blood stem cells as a control. They found no significant difference in Notch-1-IC expression in both groups and significantly higher expression levels of Jagged-1 and Dll-1 in AML blasts than in control group". (Czemerska et al, 2014) However, they performed their study on bone marrow leukemic blasts not peripheral blood blast as we did.

On breakdown of relationship between expression levels and FAB classes, we found non-significant difference in protein expression of the 3 markers relative to different FAB classes.

In their flow cytometric study, Takam et al examined protein expression in blast samples collected from 79 newly diagnosed AML patients. They reported much higher Notch-1 and Jagged-1 expression rates (85.51% and 86.08%, respectively) and much lower Dll-1 expression rate (5.80%). (Takam et al, 2019). This difference in expression level could be explained by higher presentation of M0-M1 FAB subtypes (17.22%) relative to this study (6%). It is believed that these less mature subtypes are associated with protein overexpression as documented by Sliwa et al., who found significant correlation between hyperexpression of Notch-1 and the morphological subgroups M0-M1. (Silwa et al, 2014)

In this study we evaluated the relationship between protein markers expression and different laboratory data, such as Hemoglobin level, total leukocytic count, platelet count, peripheral and bone marrow blast counts, serum enzyme (ALT, AST and LDH) and serum uric acid level. Of these parameters, only significant relationship was found between positive Notch-1 expression and significantly higher leukocytic count (60.10 ± 24.83 $x10^9$/L), however all other relationships were proven to be insignificant.

Czemerska et al reported non-significant correlations between Notch-1, Jagged-1 and Dll-1 proteins expression levels and the same laboratory data. (Czemerska et al, 2014) On the other hand, Takam et al. reported negative association of TLC with Notch-1 expression as well as positive associations between platelet count with Notch-1 and Hb level with Jagged-1. (Takam et al, 2019)

In this study, Notch-1 expression was linked with poor clinical outcome. Only 20 % of Notch-1 positive cases (2 patients) had complete remission. However, complete remission was linked to significantly lower mean Notch-1 level (15.33 ± 7.99 %) compared to non-remission cases (33.83 ± 25.38 %). Additionally, relapsed cases expressed significantly higher levels of Notch-1 compared to non-relapsed ones (38.27 ± 27.81 % vs. 20.83 ± 16.68 %), Although there was better OS and DFS in negative than in positive Notch-1 expressoin, yet the difference was not reaching statistical significance.

Our results were in agreement with those of Czemreska et al., who described that high Notch-1 expression was associated with lower but non-significant CR rates (76%) compared to low Notch-1 expressoin (58%). (Czemerska et al, 2014).

Various other reports have provided similar evidence that high levels of Notch-1 are associated with poor prognosis of AML cases. Aref et al. studied Notch-1 gene mutations in bone marrow samples obtained from 50 AML patients. They reported that unmutated cases had significantly higher CR rate (77%) and longer OS (21.2 months) compared to the mutated ones (0% and 1.2 months, respectively). (Aref et al, 2020)

Moreover, Sliwa et al., in their retrospective study used immunohistochemical analysis of 97 AML bone marrow biopsies. They found that cases with Notch-1 protein overexpression in blast cells had a significant inferior OS and 1-year survival (14%) rates compared to cases without Notch-1 hyperexpression (48%) (Silwa et al, 2014). Xu et al. also assessed the prognostic value of Notch-1 expression in bone marrow mononuclear cells using real-time PCR. They reported significantly shorter relapse-free and overall rates (8.3 ± 1.9 and 22.8 ± 2.6 months, respectively) in the patients with higher Notch-1 expression relative to those with low Notch-1 expression (13.8 ± 2.5 and 38.7 ± 3.3 months). (Xu et al, 2011)

In contrast with Notch-1, positive Jagged-1 expression in AML patients was found to be related to good prognosis. Complete remission was associated with significantly higher Jagged-1 expression (53.43 ± 25.53 %) in comparison with cases that did not show CR (26.45 ± 17.79%). Meanwhile, positive Jagged-1 expressors (n: 28) experienced relapse in 6 patients only (21.43%) and the remaining 22 (78.57%) had no relapse. Moreover, the

relapsed cases had significantly lower expression levels of Jagged-1 compared to non-relapsed cases (24.53 ± 17.50% vs. 43.46 ± 25.85%, respectively). However, there was no significant difference in OS and DFS between positive and negative Jadd-1 expressors.

Similar findings were reported by Czemreska et al. who demonstrated that CR rate of the high Jagged-1 expressors was higher than low expressors (70% vs. 52%). But unlike us, they found a significant association between positive Jagged-1 expression and increased overall survival rate (Czemerska et al, 2014). Nevertheless, different results were obtained Xu et al. who reported that higher Jagged-1 gene expression was associated with significantly shorter DFS (9.8 ± 1.3 months vs. 13.2 ± 1.7 months) and OS rates (24.6 ± 3.5 months vs. 38.5 ± 2.8 months) compared to cases with lower Jagged-1 expression (48%) (Xu et al, 2011). This discrepancy could be explained by the different methodology adopted in Xu et al. study (real-time PCR for unselected bone marrow mononuclear cells) making a room for sample contamination by non-leukemic BM cells and thus influencing PCR results.

According to our study, we failed to detect significant association between clinical outcome (CR or relapse) and Dll-1 expression levels despite the strong tendency of Dll-1 positive cases to avoid relapse (only 3 of 12 Dll-1 positive cases had relapse attack).

Other studies showed some controversial results regarding Dll-1 expression. Czemreska et al. reported non-significant lower CR rate with high Dll-1 expression (53% vs. 63%) with no relationship found between survival and Dll-1 expression (Czemerska et al, 2014). Conversely, Xu et al. found significant decrease in DFS and OS rates with high rather than low Dll-1 expression (9.9 ± 1.0 months vs. 13.6 ± 2.1 months and 24.1 ± 3.6 months vs. 38.6 ± 3.2 months, respectively) (Xu et al, 2011). It is to be noted that Xu and colleagues detected high Dll-1 expression in 54% of cases compared to 30% positive Dll-1 cases in our study.

“Our results suggest an important role of Notch-1 and Jagged-1 in the treatment response and outcome. Which might serve as a potential therapeutic target. Gu et al reported that AML cell growth arrest and caspase-dependent apoptosis could be induced through

activation of each of the four Notch receptors. suggesting the potential therapeutic use of Notch agonists in the treatment of AML" (Gu et al, 2016). "While Takam et al reported that pan-inhibition of Notch using the Notch transcription factor inhibitor SAHM1, reduced AML cell proliferation without any effect on cell death" (Takam et al, 2016)

Conclusion

Negative Notch-1 and positive Jagged-1 are associated with better treatment response and outcome in AML patients, which favorites using them as potential prognostic biomarkers in AML. Furthermore, they may be targeted for treatment of AML.

Ethical Approval and Consent :

All subjects provided a signed informed consent. Research methods were approved by Tanta Faculty of Medicine Ethics Committee, with approval number 30673/12/15. The research was done in line with the declaration of Helsinki (1964) and revised in 2008.

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